REMARKS

Claims 1 and 41-99 are pending in this application. Claims 73-75 are withdrawn from consideration, notwithstanding the fact that claim 73 appears to have inadvertently been included among the rejected claims. Claims 1 and 66 have been amended to clarify that the emulsion of step a is substantially free of a structure builder selected from the group consisting of cholesterol or cyanacrylate. This amendment is supported throughout the specification and particularly at paragraphs 0012-0014.Claims 45 and 61 have been amended to correct the lack of punctuation at the end of the sentences. Claims 55 and 66 were amended to correct a typographical error. Claim 78 was amended to delete an extraneous letter. Claim 95 was amended to delete a repeated word. No new matter has been added.

Information Disclosure Statements

Applicant is grateful for the Examiner's consideration of the references previously cited in the Information Disclosure Statement (IDS) filed on **8/2/05**. The references were considered after Applicant re-submitted an *identical* IDS along with provisions of the copies of the foreign references. NOTE: This IDS has been identified as being submitted on 8/3/09, but in fact was submitted on 8/2/05.

Regarding the Supplemental IDS filed on **8/27/07** – Applicant is also grateful to the Examiner for having considered this IDS and the references cited therein.

Double Patenting Rejections

Claims 1, 41-72 and 76-99 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting over claims 10-13 of co-pending Application No. 11/202,008.

Claims 1, 41-72 and 76-99 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting over claims 35-39 of co-pending Application No. 10/584,327.

Claims 1, 41-72 and 76-99 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting over claims 38-42 of co-pending Application No. 10/584,382.

Claims 1, 41-72 and 76-99 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting over claims 1-35 of copending Application No. 11/641,289.

Claims 1, 41-72 and 76-99 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting over claims 1-35 of copending Application No. 11/660,188.

Applicants request that these provisional double patenting rejections be held in abeyance until otherwise allowable subject matter has been reached.

Claim Objections

Claims 45 and 61 were objected to due to the lack of punctuation at the end of the sentences. Applicant has amended claims 45 and 61 to include the necessary punctuation rendering the objections moot.

Rejections Under 35 U.S.C. § 103

Applicants are grateful for the withdrawal of the rejection of claims 41-72 and 76-99 under 35 U.S.C. 103(a) for alleged obviousness over Dugstad *et al.* (US 6,221,337) ("Dugstad").

Rejections over Unger

The rejection of claims 1, 41-72 and 76-99 under 35 U.S.C. 103(a) for alleged obviousness over Unger (WO 98/42384) was maintained. As noted in Applicants prior response the Office Action cites "Unger (WO 98/04074)", but that this appears to be incorrect as Unger is not an inventor of WO/04074. Please confirm that WO 98/42384 is the correct reference.

The Examiner rejected Applicants argument that Unger fails to teach or suggest lyophilization of a lipid-containing aqueous organic emulsion. The Examiner asserts that Unger at page 50 lines 29 et seq "provides adequate motivation for including a lyoprotectant and performing lyophilization (i.e. to improve shelf life upon storage)" OA at p. 4. The Examiner also maintains that "the broader teaching of Unger teaches that a variety of fluorocarbons may be employed, including higher boiling perfluorocarbons" thus the perfluorocarbon would not be evaporated in all formulations. OA at p. 4-5.

Applicants respectfully submit that Unger cannot render the pending claims obvious. As the Examiner recognizes, none of the Unger Examples teaches or suggests the claimed lyophilization of an aqueous–organic emulsion. Example 9 discloses addition of perfluorohexane to a mixture of lipids and $CaCl_2$ in an aqueous solution. It neither teaches nor suggests a lyophilization step. Example 10 refers to Example 9 and says that 1-bromoperfluorobutance is added to the lipid mixture before the heating and sonication step and then the compositions are lyophilized, stored under a gas such as air, perfluoropropane or sulfur hexafluoride. Thus, the 1-bromoperfluorobutance, which has **a boiling point of 43°C**, as indicated in table 1 on page 8, line 7 of WO 97/40679 (a patent application of the same applicant) is added to the lipid mixture before the heating and sonication step and according to Example 9 (page 65, lines 15-16), the mixture is **heated to 45°C-50°C for one hour**. It is thus apparent that this heating treatment would inevitably result in the evaporation of the added 1-bromoperfluorobutane, having a boiling point lower than the temperature of the heated mixture. The result is that the subsequent lyophilization step of prophetic Example 10 is performed on an aqueous suspension of the lipids and not on an aqueous/organic emulsion of the lipids, as required instead by the present invention.

Applicants submit that the Examiner's assertion that one skilled in the art would be motivated to add a lyophilization step to, for instance, Example 9 is flawed. It is clear from the summary of the invention of Unger that Unger's invention is directed to a composition comprising **a gaseous precursor capable of undergoing phase transition to a gas in vivo** in a region of elevated temperature in a patient

(p. 2, lines 26-27 and p. 3, lines 2-4, 10-12 and 17-19).[1] However, a lyophilization step would necessarily cause a phase transition of the gaseous precursor to a gas during production. Thus, inclusion of a lyophilization step in the processes disclosed by Unger would preclude a phase transition in vivo, the very heart of Unger's invention. Consequently, where, as here, the actual objective of the invention is to obtain a composition including a gaseous precursor, the skilled person would have had no motivation to apply a lyophilization step to the composition obtained according to Example 9, as this would inevitably remove the main component (the gaseous precursor) of the composition. Only by applying hindsight in view of the teaching of the present invention, would one think of lyophilizing the composition obtained in example 9 of Unger.

Similarly, contrary to the Examiner's assertion, Unger's broad teachings regarding fluorocarbons would not motivate the skilled person to substitute a different fluorocarbon in Example 10. As discussed, above, Unger is directed to compositions comprising a gaseous precursor which undergo a phase shift to a gas in vivo. Example 10 clearly results in the removal of the 1-bromoperfluoropentane (BPFP) from the lipid mixture **before** the lyophilization of the aqueous suspension. According to the Unger's invention, the resulting lipid aggregate is then contacted with air or an insoluble gas. One skilled in the art would not be motivated to substitute higher boiling point fluorocarbons and then to lyophilize as the stated goal of Unger is to prepare compositions comprising gaseous precursors. As explained above, lyophilizing a composition containing even a higher boiling fluorocarbon results in a dry residue in which the gas precursor has undergone a phase transition to the gas phase during said lyophilization and thus has been removed from the composition. Thus, the composition would no longer be suitable to undergo a phase transition in vivo, the heart of Unger's invention. In sum, Unger neither teaches nor provides motivation to lyophilize an aqueous/organic emulsion as required by the instant claims. Indeed, in requiring compositions comprising a gaseous precursor Unger teaches way from the instant claims.

Moreover, lyophilization of an aqueous/organic emulsion of lipids is a key step in the claimed method of independent claims 1 and 66 and permits the production of suspensions of microbubbles having a relatively small diameter and a narrow size definition. Unger completely fails to teach or suggest this step or its advantages. Indeed, as the Examiner concedes, Unger teaches that lyophilization is used to improve storage stability and extend shelf-life. OA at p. 4. Unger neither teaches nor suggests that use of lyophilization during the process can improve the diameter and size distribution of the microbubble suspensions ultimately produced and certainly neither teaches nor suggests that **lyophilizing**

[1] Gaseous precursors are listed on page 14, line 17 et seq. They are defined on page 12, line 13 et seq as "preferably a liquid at the temperature of manufacture or storage but forms a gas at an elevated temperature under physiological conditions (such as in regions of elevated temperature of a patient. However, compounds which are solid at the temperature of manufacture or storage, but which form a gas at an elevated temperature under physiological conditions, are also within the scope of the invention."

an aqueous-organic emulsion confers these advantages. Indeed, Unger states that gas–filled vesicles prepared according to the disclosed methods "can range in size from less than about 1 μm to greater than about 100 μm." See e.g., p. 37, lines 23-25. In order to obtain microvesicles of smaller diameter and narrower size distribution Unger teaches use of extrusion or filtration. See e.g., p. 37, line 19 – p. 38, line 25. The claimed process obviates the need for these additional, time and resource-consuming steps, advantages neither taught nor suggested by Unger.

Applicant notes that in view of the failure of Unger to disclose a critical element of independent claims 1 and 66, this reference fails to render unpatentable the dependent claims for at least this reason.

Rejections Over Linder in View of Unger and Dugstad

Claims 1, 41-72 and 76-99 were rejected under 35 U.S.C. 103(a) as being unpatentable over Linder (WO 94/01140, "Linder") in view of Unger (WO 98/04074, "Unger") and Dugstad *et al.* (US 6,221,337, "Dugstad") Although the Examiner admits that Linder "does not specifically teach at least that the amphiphilic material of the emulsifying composition comprises more than 50% by weight of phospholipid, the Examiner asserts that it would have been obvious "to modify the ratio of phospholipid as a matter of routine experimentation in preparation of reconstitutable composition for producing microbubble contrast agents," particularly "because Unger and Dugstad teaches preparation of phospholipid vesicles comprising varying surfactant ratios, including those comprising predominantly phospholipid membranes." Applicants respectfully traverse.

Linder

As explained in the instant specification at paragraphs 0012-0014, Linder is directed to a process for preparing microvesicle suspensions by lyophilizing aqueous emulsions containing emulsifiers, non-polar liquids and lipid-soluble or water insoluble "structure builders" and reconstituting the lyophilized product. See Linder, p. 2, 2nd paragraph. The structure builders, which are preferably cholesterol or cyanacrylate, are present in the reaction mixture in amounts from 0.05-10% and preferably 1-5%. As explained in paragraphs 0013 and 0014 of the US publication of the instant application, and particularly in Example 10, Applicants found that the presence of structure builders negatively affects both the conversion yield and the size distribution of the microbubbles suspensions obtained. The currently pending claims exclude the presence of such materials in the aqueous-organic emulsion, which is lyophilized. Thus, Linder's requirement of structure builders clearly teaches away from the claimed invention.

Additionally, phospholipids are always employed in combination with poloxamers in the working examples of Linder. Thus, the amount of phospholipids in the reaction mixture is much lower than the claimed 50% of the present invention (e.g. Example 1 – 30 g poloxamer, 30 g phosphatidylglycerol, 20 g

cholesterol= 37.5% phospholipid; Example 2 – 35 g poloxamer, 25 g dimyristoylphosphatidylglycerol, 10 g cholesterol-35% phospholipid).

For at least these reasons, Linder fails to teach or suggest all elements of the claimed invention and in fact teaches away as Applicants have shown that emulsions containing structure builders provide unsatisfactory results.

The Secondary References Unger and Dugstad

The Examiner asserts that Unger and Dugstad would have provided motivation and an expectation of success to modify the ration of phospholipid/poloxamer in the lyophilisate in Linder to provide greater than 50% by weight phospholipid. Applicants respectfully disagree. Even if one were to assume that Unger and Dugstad suggest use of at least 50% phospholipid, substituting this element in the process of Linder does not provide the claimed invention given Linder's requirement of structure builders, which Applicants have established provide unsatisfactory results.

Moreover, as explained above and in Applicants last response, Unger and Dugstad fail to teach or suggest the claimed step of lyophilizing an aqueous-organic emulsion; thus no combination of these references teaches or suggests each element of the claimed invention.

Applicants respectfully maintain that claims 1, 41-72 and 76-99 are in condition for allowance, and request the issuance of a notice of allowance with respect to the same.

No fee is believed to be due with the filing of this Amendment and Response to Office Action. However, if any additional fee is necessary, applicant hereby authorizes such fee to be charged to Deposit Account No. 50-2168. If an interview with the Examiner would expedite the prosecution of this application, the Examiner is respectfully invited to contact the undersigned.

Favorable action is respectfully requested.

Respectfully submitted,

Dated: July 20, 2010

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